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The Female Urinary Microbiota in Urgency Urinary Incontinence

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Abstract

Objectives—To characterize the urinary microbiota in women planning treatment for urgency urinary incontinence and to describe clinical associations with urinary symptoms, urinary tract infection and treatment outcomes.

Study Design—Catheterized urine samples were collected from female multi-site randomized trial participants without clinical evidence of urinary tract infection and 16S rRNA gene sequencing was used to dichotomize participants as either DNA sequence-positive or sequence-negative. Associations with demographics, urinary symptoms, urinary tract infection risk, and treatment outcomes were determined. In sequence-positive samples, microbiotas were characterized on the basis of their dominant microorganisms.

Results—Over half [51.1% (93/182)] of the participants' urine samples were sequence-positive. Sequence-positive participants were younger (55.8 vs. 61.3, $p=0.0007$), had a higher body mass index (33.7 vs. 30.1, $p=0.0009$), had a higher mean baseline daily urgency urinary incontinence episodes (5.7 vs. 4.2, $p<0.0001$), responded better to treatment (decrease in urgency urinary incontinence episodes -4.4 vs. -3.3 , $p=0.0013$) and were less likely to develop urinary tract

infection (9% vs. 27%, $p=0.0011$). In sequence-positive samples, eight major bacterial clusters were identified; seven clusters were dominated by a single genus, most commonly *Lactobacillus* (45%) or *Gardnerella* (17%), but also other taxa (25%). The remaining cluster had no dominant genus (13%).

Conclusions—DNA sequencing confirmed urinary bacterial DNA in many women without signs of infection and with urgency urinary incontinence. Sequence status was associated with baseline urgency urinary incontinence episodes, treatment response and post-treatment urinary tract infection risk.

Keywords

Microbiota; Microbiome; Urinary Bacteria; Urgency Urinary Incontinence; Urinary tract infection; Cystitis

Introduction

Urgency urinary incontinence (UUI) is a heterogeneous condition usually attributed to abnormalities in detrusor neuro-muscular functioning and/or signaling. However, most incontinence experts suspect that UUI etiology is likely more complex. Current first line treatment for UUI is behavioral and/or pharmacologic, but many affected patients have persistent symptoms. Thus, the group of women affected by the generic symptom of UUI may have multiple, heterogeneous etiologies.

The Anticholinergic Versus Botulinum Toxin A Comparison (ABC) Trial was a 10-center, double-blind, double-placebo-controlled randomized trial in which women received either one intradetrusor injection of 100 U of onabotulinumtoxin A and daily oral placebo or daily oral anticholinergic medication and one intra-detrusor injection of saline¹. The ABC study was designed and conducted prior to knowledge that the female lower urinary tract (bladder and/or urethra) contains bacterial communities (termed the female urinary microbiome).

Growing evidence suggests that the female urinary microbiota may play a role in certain urinary disorders such as UUI². Our research team previously described the female urinary microbiota of women with UUI³ using catheterized urine samples obtained at baseline from patientparticipants in a randomized clinical trial for the treatment of UUI who did not have clinical urinary tract infection (UTI)¹. Previously, we also compared various techniques to verify that transurethral catheterization was an appropriate method to collect urine samples with minimal vulvo-vaginal contamination⁴. This current analysis goes beyond the initial testing of the prior samples to formally characterize the female urinary microbiota by 16S rRNA gene sequence analysis. Relationships between sequence status, microbiota characteristics and clinical variables of interest, including treatment response and post-treatment UTI risk, were also assessed.

Materials and Methods

Subjects and Specimen Acquisition

The full methods of the trial and the primary outcome of the ABC trial have been published^{1, 5}. Briefly, the trial randomized women without neurologic disease with moderate-to-severe UUI, defined as having ≥ 5 episodes of UUI per 3-day period. Participants were anticholinergic drug naive or had previously used ≤ 2 anticholinergic medications other than the study drugs. Exclusion criteria included a post-void residual volume ≥ 150 mL or previous therapy with oral study medications or onabotulinumtoxin A.

The primary outcome was reduction from baseline in mean episodes of urgency urinary incontinence (UUIE) per day over the 6-month period, as recorded in 3-day diaries submitted monthly. Secondary outcomes included resolution of UUI symptoms, quality of life, use of catheters, and adverse events including UTI, which was defined as a positive standard urine culture with $>10^5$ colony-forming units of a known uropathogen or treatment with antibiotic for UTI within 6 months of randomization. Following treatment, intermittent catheterization was recommended if post-void residual was either $>300\text{mL}$ or $>150\text{mL}$ with “moderate” to “quite a bit” of bother associated with incomplete voiding.

All subjects were free of clinical UTI prior to study injection, as determined by a negative nitrite and leukocyte esterase result on a urine dipstick evaluation of a catheterized specimen. Urine culture was not required. Following baseline assessment and prior to injection, subjects provided a baseline catheterized urine sample, which was placed at -80°C within 1 hour; the frozen samples were batch-shipped on dry ice to Loyola University Chicago for 16S rRNA gene sequence analysis. Staff and investigators were blinded to clinical information during laboratory research analyses.

16S rRNA Sequencing

DNA was isolated in a laminar flow hood to avoid contamination. Genomic DNA was extracted from 1 ml of urine using previously validated protocols^{6–8}. Variable region 4 (V4) of the bacterial 16S rRNA gene was amplified via a two-step nested polymerase chain reaction (PCR) protocol, using modified universal primers 515F and 806R, as previously described^{6, 87}. Two quality controls assessed the contribution of extraneous DNA from laboratory reagents: a DNA extraction negative control with no urine added and a PCR negative control with no template DNA added. The final PCR product was purified from unincorporated nucleotides and primers using Qiaquick PCR purification kit (Qiagen, Valencia, CA) and Agencourt AMPure XP-PCR magnetic beads (Beckman coulter). Samples were normalized to equal DNA concentration, as determined by Nanodrop spectroscopy (Thermo Scientific, Waltham, MA). The sample library and the PhiX sequencing control library (Illumina) were denatured and added to the 2×250 bp sequencing reagent cartridge, according to manufacturer’s instructions (Illumina).

DNA Sequence Analysis

Illumina MiSeq post-sequencing software preprocessed sequences, removing primers and sample indices. The open-source program mothur (v1.31.2) combined paired end reads and

removed contigs (overlapping sequence data) of incorrect length (<285 bp, ≥100 bp) and/or contigs containing ambiguous bases⁹. The remaining modified sequences were aligned to the SILVA reference database¹⁰. Chimeric sequences were removed using UCHIME. The remaining sequences were taxonomically classified using a naïve Bayesian classifier and the RDP training set v9, and clustered into operational taxonomic units (OTUs)¹¹. METAGENassist was used to link OTU nomenclature to taxonomic assignments¹².

The ABC trial was conducted with full IRB approval at all participating sites. All samples were processed in duplicate and characterized as sequence-positive, sequence-negative or inconclusive. A sequence-negative sample was one in which DNA was not amplified in either technical replica. To avoid accidentally sequencing rare contaminants, we conservatively considered samples to be negative if DNA amplification was not visible on an agarose gel. A sequence-positive sample was one in which DNA was amplified from both replicas, the replicas had a Euclidian distance score less than 0.3, and the dominant genus (>45% sequences per sample), if present, was the same in both replicas. Samples not meeting these criteria were deemed inconclusive and disregarded from further analysis (n=12). For each sequence-positive sample, percent reads were calculated and replicates were then averaged for downstream analysis.

To display the average sequence abundances, a histogram was produced, color-coded by bacterial taxa. Euclidean distance was calculated between samples and the complete method was used for hierarchical clustering in the statistical package R v2.15.1. The resulting dendrogram was divided into 8 urotypes⁷. The rare urotypes were grouped into an “other” category, condensing the original 8 urotypes to a total of 5 urotypes for use in the analysis (*Lactobacillus*, *Gardnerella*, *Gardnerella/Prevotella*, *Diverse*, and *Other*) plus the negative category. These urotypes, along with the sequence-negative group, were then compared to participant demographics, symptoms at baseline and clinical outcomes.

Statistical Analysis

Differences were examined descriptively at baseline, as were changes in clinical outcome measures for individuals defined as sequence-positive and sequence-negative, as well as for individuals classified by urotype, further described below. The differences in binary baseline and outcome measures across these two categorical measures of sequence were examined via contingency tables, with p values generated from Chi-squared tests and differences in mean values for continuous measures evaluated using general linear models. Because all analyses were considered descriptive, no adjustments were made for multiple comparisons and p values should be interpreted accordingly. To compare the mean abundance of the top 10 most abundant taxa, the Wilcoxon rank sum test was performed. Statistical analyses were conducted using SAS version 9.3.

Results

Approximately half (51.1%, 93/182) the urine samples were sequence-positive. Table 1 displays demographics and baseline characteristics of participants relative to sequence status; the mean age was 58.5 years and most participants were white (77%). Sequence-positive subjects were younger (55.8 ± 12.2 vs. 61.3 ± 9.0 years, p=0.0007), had a higher

body mass index (33.7 $\pm$ 7.3 vs. 30.1 $\pm$ 6.6 kg/m², $p=0.0009$) and had a higher mean number of baseline UIIE (5.7 $\pm$ 2.5 vs. 4.2 $\pm$ 2.1 per day, $p<0.0001$). Race, ethnicity, prior anticholinergic use, or study treatment assignment did not differ between sequence-positive and sequence-negative cohorts.

Sequence-positive subjects responded better to treatment with a larger decrease in baseline UIIE (-4.4 ± 2.7 vs. -3.3 ± 1.9 , $p=0.0013$) with no evidence of interaction with treatment group ($p=0.92$). In both groups, sequence-positive subjects were less likely to develop UTI after initiation of study anti-incontinence treatment (9% vs. 27% post treatment UTI's, $p=0.0011$) (Table 2).

In sequence-positive urines, hierarchical clustering revealed eight major urotypes, as shown by the horizontal line (Figure 1, **top**). In seven of these urotypes, a single bacterial genus or family dominated (i.e., represented $>45\%$ total sequences in a sample) (Figure 1, **bottom**). For example, in the first urotype, Enterobacteriaceae accounted for $>90\%$ of the total sequences in all of the eight samples. Nearly half of the sequence positive urines were dominated by the genus *Lactobacillus* (45%), followed by *Gardnerella* (17%), *Gardnerella/Prevotella* (9%), Enterobacteriaceae (9%), *Staphylococcus* (3%), *Aerococcus* (2%) and *Bifidobacterium* (2%). The remaining cluster was labeled "Diverse" to signify those (13%) without a dominant genus. While these more diverse samples were often composed of different genera, they grouped together (Table 1, **bottom**). Each urotype was observed in samples from 2 performance sites and several urotypes were observed at multiple clinical sites (Table 3).

Most sequence-positive samples had a dominant genus (Figure 1). To assess the prevalence of these genera in samples where they did not dominate, we calculated the proportion of sequences belonging to the dominant taxon of each urotype across all sequence-positive samples. Figure 2 demonstrates that nearly all the sequence-positive samples contained *Lactobacillus* (with a median 20% *Lactobacillus* sequences per urine sample). With the exception of *Gardnerella*, the other urotype-defining taxa were less commonly detected in samples outside those they dominated.

Cluster analysis (Figure 1) revealed eight major urotypes: *Lactobacillus*, *Gardnerella*, *Gardnerella/Prevotella*, Enterobacteriaceae, *Staphylococcus*, *Aerococcus*, *Bifidobacterium*, and Diverse. Although some major clusters (especially *Lactobacillus*, *Gardnerella* and Diverse) branched into sub-clusters, for further analyses, we treated these sub-clusters as one urotype. We also combined the less common urotypes (*Gardnerella/Prevotella*, Enterobacteriaceae, *Staphylococcus*, *Aerococcus* and *Bifidobacterium*) together (Other). We compared these five urotypes and the sequence-negative group to baseline demographic and clinical variables. Whereas race, ethnicity and menopausal status were similar across urotypes, age ($p=0.005$) and body mass index ($p=0.014$) differed (Table 4). Additional analysis detected an upward trend for age with the Enterobacteriaceae-dominant and *Staphylococcus*-dominant urotypes and a downward trend with the *Gardnerella/Prevotella*-dominant urotype (Figure 3A). Body mass index tended to be lower in the Enterobacteriaceae-dominant, *Staphylococcus*-dominant and sequence negative groups (Figure 3B). Whereas symptom severity was similar across urotypes, baseline UIIE differed

by incontinence severity ($p=0.046$) and frequency ($p<0.0001$) (Table 4). While *Staphylococcus*-dominant and sequence-negative groups showed a trend towards lower baseline UUIE (Figure 3C), the number of urine samples representing these urotypes was small and the analysis lacked statistical power. Treatment response (change in UUIE, $p=0.0017$) and development of post-treatment UTI ($p=0.0058$) differed among the 5 urotypes (Table 5).

We compared the mean sequence abundance of sequence-positive participants by clinical variables. The two treatment cohorts (anticholinergic and onabotulinumtoxin A) displayed very similar mean sequence profiles at baseline, as predicted given randomized treatment assignment (Figure 4). However, the mean sequence profile of the sequence-positive women who developed a post-treatment UTI differed from those who did not develop a UTI. On average, sequence-positive women who developed a post-treatment UTI had fewer *Lactobacillus* sequences (14 vs. 46%, $p=0.009$) (Figures 4 and 5).

Comment

Principal findings of the study

In adult women with UUI, the composition of the urinary microbiota is identifiable, variable and related to certain clinical variables of potential importance. As the female urinary microbiota has only recently been detected^{3, 4, 6, 7, 13–15}, an expanded perspective is warranted. The current study demonstrates that the status of the female urinary microbiota (sequence-positive or sequence-negative) can be used to sub-classify women with UUI and that sequence-positive women can be further sorted by composition into urotypes, based on the dominant bacterial genus or family or the lack thereof. The clinical utility of such sorting is yet to be established. However, the likelihood that UUI is a heterogeneous urinary disorder could make this new approach useful for patient phenotyping.

Meaning of the findings

Although the ABC study was designed prior to knowledge of the female urinary microbiota and our analysis was not based on an *a priori* hypothesis, the current findings add to the growing evidence that document the existence and define the characteristics of the female urinary microbiota^{3, 4, 6, 7, 13–15}. Further study of the differences in biomass (sequence positive vs. sequence negative) and the microbial diversity by specific and dominant organisms is likely to advance our knowledge of UUI. The composition of the urinary microbiota and its clinical relationships also requires further study in other clinically relevant groups, including women undergoing surgery for prolapse and/or stress urinary incontinence, and those without lower urinary tract symptoms.

Clinical Implications

To understand potential clinical relationships with the female urinary microbiota, studies of comparison populations are clearly needed, including asymptomatic women and those affected by non-UUI urinary symptoms. In an unrelated, smaller study, we compared the female urinary microbiota of adult women with and without UUI⁷. The results of this previous study closely align with those of the current one. Approximately half the samples

were sequence-positive and most of those were dominated by one genus, with the *Lactobacillus* and *Gardnerella* urotypes most common. However, several species were strongly associated with UI. These included emerging uropathogens (e.g., *Aerococcus urinae*), *Gardnerella vaginalis*, and *Lactobacillus gasseri*. In contrast, *Lactobacillus crispatus* was associated with women without lower urinary tract symptoms.

The bladder is an environment with low bacterial abundance, unlike the gut or the vagina. Although bacterial DNA was not sequenced from approximately half of our participants' urine samples, it is possible that these sequence-negative bladders are not sterile, but rather contain bacteria at low difficult-to-sequence levels. The existence of this sequence-negative status is clinically important, as this group was at significantly greater risk for post-treatment UTI. This finding will require further study; it is possible that certain organisms, such as *Lactobacillus*, play an important regulatory or protective role to challenge the growth of common uropathogens, such as *E. coli*.

Research implications

Combined with the extreme sensitivity of PCR, caution is needed in low abundance environments to appropriately distinguish true members of the urinary microbiota from rare contaminants. Thus, we conservatively considered samples that amplified weakly to be sequence-negative to avoid over-interpretation of microbiota components. Even so, it is notable that the improved methods used in the current study reproducibly detected bacterial DNA in the urine of 56% of women in this population, compared to 39% in our previous analysis³.

DNA-based techniques are increasingly popular for microbiota study because they can detect bacteria without culture. Indeed, several groups have used 16S rRNA gene sequence analysis to detect the microbiota in the lower urinary tract of adult women and men^{4, 6, 7, 13, 15–18}. Clinical reliance on standard urine cultures has limited our consideration of bacteria as etiological contributors to idiopathic lower urinary tract disorders, including UI. This is because standard urine culture techniques underestimate the composition and diversity of bacteria, especially those that inhabit the lower urinary tract. Expanded urine culture techniques have been recently developed and shown to significantly outperform the standard techniques^{6, 14}, but these new methods were developed after the samples in this study were obtained. Importantly, recent studies using our newly developed expanded quantitative urine culture (EQUC) method have documented that bacteria detected by 16S rRNA sequencing are alive and thus the sequencing is not simply detecting DNA of dead organisms^{6, 7}.

Strengths and weaknesses

Some investigators may consider our analysis limited because our operational definition of “clinically free of UTI” used a negative dipstick, rather than require that they have a negative standard urine culture at baseline. Given the significant limitations of standard urine culture^{6, 7, 14}, however, we do not consider this a major limitation for the ABC study or this analysis. Furthermore, few samples included uropathogens, typically detected by standard urine culture (e.g., *E. coli*) that could be indicative of a UTI. Given the nature of

the index study, which was aimed at clinical treatment of affected patients, there is no age-matched control population without UUI symptoms in this randomized trial. As one of the first studies of the female urinary microbiota, it is underpowered to definitively assess differences in the distribution of the urinary microbiota by race or certain other variables.

Conclusion

Women with UUI are a heterogeneous population on the basis of their baseline urine bacterial sequence status and/or urotype. Nearly half of female trial participants with UUI, without evidence of clinical infection, had a urotype that was typically dominated by a single genus, most often *Lactobacillus* or *Gardnerella*. These bacterial communities appear to have clinical relationships with baseline UUI symptoms, response to treatment and risk for post-treatment UTI. As research in this area expands and incorporates samples from other clinically relevant populations, we fully anticipate further refinements in our understanding of the role of the female urinary microbiota in health and disease. Our findings suggest that previously undetected bacteria in the bladder of women may have a role in UUI, providing expanded opportunities for prevention and improved treatment approaches for UUI, such as modification of the microbiota to improve response to UUI treatments. Our findings also suggest that some bacteria may play a protective role in the bladder as they do in other human biological niches. The potential for such a protective role warrants further study.

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Figure 1. The urinary microbiota profile of sequence positive participants

The urinary microbiota profiles of sequence positive participants cluster together, as demonstrated in the dendrogram (top), and by the dominant bacterial taxa present, as depicted in the histogram (bottom). The dendrogram was based on clustering of the Euclidean distance between urine samples and each line represents a separate individual. Urine samples that possessed the same dominant bacterial taxa grouped together in the dendrogram and were classified into the following urotypes, as shown by the dashed horizontal line: *Lactobacillus*, *Gardnerella*, *Gardnerella/Prevotella*, *Enterobacteriaceae*,

Staphylococcus, *Aerococcus* and Diverse. The placement of the urotype grouping line provided clear distinction of urine samples by the dominant genera, while maintaining clusters that contain at least 2 urine samples. The histogram displays the bacterial taxa detected by sequencing as the percentage of sequences per urine by sequence positive participants (N=93). Each bar on the x-axis represents the urinary microbiota sequence-based composition of a single participant. The y-axis represents the percentage of sequences per participant with each color corresponding to a particular bacterial taxon. Bacteria were classified to the genus level with the exception of Enterobacteriaceae and Lachnospiraceae, which could only be classified to the family level. The 15 most sequence abundant bacterial taxa were displayed and the remainder of the taxa, including unclassified sequences, were grouped into the category “Other”.

Figure 2. Distribution of dominant taxa in urine of all sequence positive participants

The sequence proportion of dominant taxa (taxa that accounted for greater than 45% of the sequences in at least one sample) were graphed for the sequence positive samples (N=93). The boxplots represent the 25th, 50th and 75th percentile of the sequence proportion, while points represent outliers. *Lactobacillus* was detected in the majority of urine samples and the sequence abundance ranged from 0 to 100% of the total sequences per sample. The median amount of *Lactobacillus* sequences detected per urine was 20%. *Gardnerella* was the second most frequently detected genus, with 43% of samples containing >1% *Gardnerella* sequences. Whereas *Staphylococcus*, *Aerococcus*, Enterobacteriaceae and *Bifidobacterium* were detected in high abundance in a few samples, they were present at very low levels or not at all in the remainder of samples. For example, *Staphylococcus* and *Aerococcus* were detected at >45% of total sequences in only 3 and 2 samples, respectively. Lacto, *Lactobacillus*; Gard, *Gardnerella*; Prev, *Prevotella*; Staph, *Staphylococcus*; Aero, *Aerococcus*, Entero, Enterobacteriaceae, and Bifido, *Bifidobacterium*.

Figure 3. Demographic variable distribution by urotype, including the sequence-negative group
 Boxplots are shown comparing each urotype and the sequence negative group to A) age, B) weight, BMI and C) the number of UI episodes at baseline for each participant.
 Aero=Aerococcus, Bif=Bifidobacterium, Ent=Enterobacteriaceae, Gard=Gardnerella, Lac=Lactobacillus, Div = diverse, G/P =Gardnerella/Prevotella, Staph=Staphylococcus and Neg = sequence-negative group. N stands for the number of samples within each group.

Figure 4. Comparison of average bacterial sequence abundance in urine by treatment group and UTI outcome

The average amount of bacterial sequences detected in the sequence positive urine of each randomized treatment cohort (anticholinergic versus botox) and UTI outcome cohort (positive versus negative) was calculated. The average bacterial sequence abundance profiles were similar between treatment cohorts, whereas the profiles differed between UTI outcome cohorts.

Figure 5. Urinary microbiota profiles by UTI outcome

The 15 most abundant bacteria detected by sequencing were displayed as the percentage of sequences per sample on the y-axis. The vertical bars along the x-axis represent the microbiota profile of individual participants separated by UTI outcome. The urinary microbiota profiles of the eight UTI positive participants consist of the following urotypes: 1 *Lactobacillus*, 1 *Gardnerella*, 2 *Enterobacteriaceae*, 1 *Staphylococcus*, 1 *Aerococcus*, 1 *Bifidobacterium* and 1 diverse. The proportion of *Lactobacillus*-dominant urines and diverse urines is less in the UTI positive group compared to the UTI negative group.

Table 1

Baseline Characteristics and Sequence Status

Characteristic	Overall	Sequence Positive	Sequence Negative	p-value
Age, N	182	93	89	0.0007
Mean (SD)		55.8 (12.2)	61.3 (9.0)	
Median (Min-Max)		55.8 (31.1–85.4)	61.2 (43.1–83.1)	
Ethnicity, N (%)				0.054
Hispanic		23 (25%)	12 (13%)	
Non-Hispanic		70 (75%)	77 (87%)	
Race, N (%)				0.15
White		68 (73%)	73 (82%)	
Non-White		25 (27%)	16 (18%)	
BMI, N		93	89	0.0009
Mean (SD)		33.7 (7.3)	30.1 (6.6)	
Median (Min-Max)		32.8 (20.5–52.4)	28.8 (18.7–48.5)	
Menopausal Status, N (%)				0.059
Pre-menopausal		17 (20%)	8 (9%)	
Post-menopausal		70 (80%)	77 (91%)	
Prior anticholinergic use, N (%)				0.97
Yes		52 (56%)	50 (26%)	
No		41 (44%)	39 (44%)	
Baseline Urgency Urinary Incontinence Episodes (UUIE) Stratum, N (%)				0.0036
5 to 8		11 (12%)	26 (29%)	
9+		82 (88%)	63 (71%)	
Baseline UUIE, N		93	89	<0.0001
Mean (SD)		5.66 (2.5)	4.20 (2.1)	
Median (Min-Max)		5.00 (1.7–12.0)	3.67 (1.7–9.7)	
OABq ^{**} Symptom Severity, N		93	89	0.78
Mean (SD)		68.1 (18.8)	68.8 (18.5)	
Median (Min-Max)		70 (16.7–100)	70 (26.7–100)	
OABq ^{**} HRQL, N		93	89	0.80
Mean (SD)		45.6 (20.7)	46.3 (24.0)	
Median (Min-Max)		47.7 (0–90.8)	46.2 (6.2–98.5)	
Treatment, N (%)				0.47
Active onabotulinumtoxin A		42(45%)	45 (51%)	
Active anticholinergic medication		51 (55%)	44 (49%)	

* p-value, testing sequence positive versus negative

** OABq: Overactive Bladder quantitative

Table 2

Clinical Outcomes by Sequence Status

Characteristic	Sequence Positive	Sequence Negative	p-value
Urinary tract infection, N (%)			0.0011
Yes	8 (9%)	24 (27%)	
No	85 (91%)	65 (73%)	
Change in UIIE, N	90	89	0.0013
Mean (SD)	-4.36 (2.7)	-3.32 (1.9)	
Median (Min-Max)	-4.03 (-11.7 - 0.7)	-3.17 (-9.3 - 2.5)	
OABq Symptom Severity Change, N	90	89	0.37
Mean (SD)	-46.8 (23.8)	-43.7 (22.7)	
Median (Min-Max)	-48.1 (-86.7 - 11.1)	-44.4 (-93.3 - 9.2)	
OABq HRQL Change, N	90	89	0.60
Mean (SD)	39.2 (21.9)	37.3 (25.2)	
Median (Min-Max)	37.5 -9.0 - 96.7)	36.9 (-28.8 - 93.8)	

Table 3

Urotype distribution among collection locations. For each urotype, we verified that the samples came from at least two study sites, to rule out bias due to the collection location.

Urotype	N	Site ID
<i>Aerococcus</i>	2	08, 22
<i>Bifidobacterium</i>	2	02, 16
Diverse	12	02,07,15,17,18,21
<i>Enterobacteriaceae</i>	8	02,06,07,17,18
<i>Gardnerella</i>	16	02,06,07,15,16,17,18,22
<i>Gardnerella/Prevotella</i>	8	02,07,08,18,22
<i>Lactobacillus</i>	42	02,06,07,14,15,16,17,18,21,22
<i>Staphylococcus</i>	3	02,17

Table 4

Baseline Characteristics as a Function of Urotype.

Characteristic	<i>Lactobacillus</i>	<i>Gardnerella</i>	Diverse	Other	Negative	p-value
Age, N	42	16	12	23	89	0.0005
Mean (SD)	53.2 (11.5)	53.7 (10.0)	61.0 (11.2)	59.5 (14.0)	61.3 (9.0)	
Median	53.5	54.2	60.3	58.4	61.2	
Ethnicity, N (%)						0.24
Hispanic	10 (24%)	5 (31%)	4 (33%)	4 (17%)	12 (13%)	
Non-Hispanic	32 (76%)	11 (69%)	8 (67%)	19 (83%)	77 (87%)	
Race, N (%)						0.20
Caucasian	28 (67%)	11 (69%)	11 (92%)	18 (78%)	73 (82%)	
Non-Caucasian	14 (33%)	5 (31%)	1 (8%)	5 (22%)	16 (18%)	
BMI, N	42	16	12	23	89	0.014
Mean (SD)	33.8 (7.6)	32.6 (5.2)	35.2 (8.7)	33.3 (7.3)	30.1 (6.6)	
Median	32.5	32.5	36.0	32.8	28.8	
Menopausal Status, N (%)						0.22
Pre-menopausal	10 (26%)	4 (22%)	2 (17%)	1 (6%)	8 (9%)	
Post-menopausal	29 (74%)	14 (78%)	10 (83%)	17 (94%)	77 (91%)	
Prior anticholinergic use, N (%)						0.71
Yes	23 (55%)	8 (50%)	9 (75%)	12 (52%)	50 (56%)	
No	19 (45%)	8 (50%)	3 (25%)	11 (48%)	39 (44%)	
Baseline UIUE Stratum, N (%)						0.046
5 to 8	6 (14%)	2 (12%)	0 (0%)	3 (13%)	26 (29%)	
9+	36 (86%)	14 (88%)	12 (100%)	20 (87%)	63 (71%)	
Baseline UIUE, N	42	16	12	23	89	<0.0001
Mean (SD)	5.0 (2.1)	6.0 (2.8)	6.2 (1.9)	6.3 (2.9)	4.2 (2.1)	
Median	4.50	5.17	6.50	6.00	3.67	
OABq Symptom Severity, N	42	16	12	23	89	0.49
Mean (SD)	65.2 (17.4)	66.6 (19.1)	69.2 (17.5)	73.8 (21.4)	68.8 (18.5)	
Median	60.0	71.67	70.0	76.7	70.0	

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Characteristic	<i>Lactobacillus</i>	<i>Gardnerella</i>	Diverse	Other	Negative	p-value
OABq HRQL, N	42	16	12	23	89	0.32
Mean (SD)	49.0 (17.7)	49.5 (21.2)	42.9 (24.1)	37.5 (22.5)	46.3 (24.0)	
Median	50.8	53.7	40.0	43.1	46.2	

Table 5

Clinical Outcomes as a Function of Urotype.

Characteristic	Lactobacillus	Gardnerella	Diverse	Other	Negative	p-value
UTI, N (%)						0.0058
Yes	1 (2%)	1 (6%)	1 (9%)	5 (22%)	24 (27%)	
No	41 (98%)	15 (94%)	11 (91%)	18 (78%)	65 (73%)	
Change in UIUE, N	42	16	12	23	89	0.0017
Mean (SD)	-3.8 (2.3)	-4.9 (3.0)	-5.2 (1.9)	-5.0 (3.1)	-3.3 (1.9)	
Median	-3.61	-4.08	-5.53	-4.90	-3.17	
OABq Symptom Severity Change, N	42	16	12	23	89	0.50
Mean (SD)	-42.7 (24.7)	-48.0 (25.2)	-51.8 (18.5)	-51.1 (24.2)	-43.7 (22.7)	
Median	-45.0	-46.7	-48.3	-54.4	-44.4	
OABq HRQL Change, N	42	16	12	23	89	0.38
Mean (SD)	35.2 (19.7)	36.4 (28.6)	48.5 (17.4)	43.8 (21.3)	37.3 (25.2)	
Median	33.5	35.3	48.5	46.4	36.9	